



**University of  
Zurich**<sup>UZH</sup>

**Zurich Open Repository and  
Archive**

University of Zurich  
University Library  
Strickhofstrasse 39  
CH-8057 Zurich  
[www.zora.uzh.ch](http://www.zora.uzh.ch)

---

Year: 2016

---

## **Current State of Immune-Based Therapies for Glioblastoma**

Lim, M ; Weller, M ; Chiocca, E A

**Abstract:** Glioblastoma is one of the most aggressive solid tumors, and, despite treatment options such as surgery, radiation, and chemotherapy, its prognosis remains grim. Novel approaches are needed to improve survival. Immunotherapy has proven efficacy for melanoma, lung cancer, and kidney cancer and is now a focus for glioblastoma. In this article, glioblastoma-mediated immunosuppression will be discussed and two exciting immune approaches, checkpoint inhibitors and viral-based therapies, will be reviewed.

DOI: [https://doi.org/10.14694/EDBK\\_159084](https://doi.org/10.14694/EDBK_159084)

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-124355>

Journal Article

Published Version

Originally published at:

Lim, M; Weller, M; Chiocca, E A (2016). Current State of Immune-Based Therapies for Glioblastoma. American Society of Clinical Oncology Educational Book, 35:e132-e139.

DOI: [https://doi.org/10.14694/EDBK\\_159084](https://doi.org/10.14694/EDBK_159084)

# Current State of Immune-Based Therapies for Glioblastoma

Michael Lim, MD, Michael Weller, MD, and E. Antonio Chiocca, MD, PhD

## OVERVIEW

**Glioblastoma is one of the most aggressive solid tumors, and, despite treatment options such as surgery, radiation, and chemotherapy, its prognosis remains grim. Novel approaches are needed to improve survival. Immunotherapy has proven efficacy for melanoma, lung cancer, and kidney cancer and is now a focus for glioblastoma. In this article, glioblastoma-mediated immunosuppression will be discussed and two exciting immune approaches, checkpoint inhibitors and viral-based therapies, will be reviewed.**

Glioblastoma has been studied as a paradigmatic tumor for cancer-associated immunosuppression for more than 3 decades. Initial observations included the characterization of decreased immune responsiveness of peripheral blood cells harvested from patients with glioblastoma.<sup>1</sup> This remote effect of a locally growing neoplasm could only be explained by soluble factors released by the tumor in sufficient quantities to induce systemic immunosuppression (Fig. 1). The search for soluble factors produced by cultured glioblastoma cells resulted in the identification of, among others, transforming growth factor (TGF)- $\beta$  as a key immunosuppressive cytokine that has remained a therapeutic target until today. Yet, local application of antisense oligonucleotides failed presumably because of poor target coverage, whereas systemic application of TGF- $\beta$  receptor antagonists has its limits in nonhematologic toxicity. TGF- $\beta$  is a member of a large family of cytokines that interacts with heterodimeric receptors. There are three TGF- $\beta$  isoforms in humans that have nonoverlapping functions, at least in development, as demonstrated in knockout mouse models. In contrast, the three isoforms are coregulated in glioblastomas, and no isotype-specific roles have been identified so far in the biology of glioblastoma.<sup>2</sup> Other soluble immunosuppressive factor candidates attributed a role in shaping the immunosuppressive microenvironment in glioblastoma include interleukin 10 and prostaglandin E2.

Admittedly, it has not been clarified definitively that the peripheral immunosuppression encountered among patients with glioblastoma is caused exclusively or even mainly by elevated levels of soluble mediators systemically. Alternatively, it is conceivable that immune modulatory cells that encounter the glioblastoma microenvironment are forced to develop into an immunosuppressive immune cell population that

indirectly mediates systemic immunosuppression. In fact, it has recently been recognized that glioblastoma cells are capable of reshaping the phenotype of tumor-infiltrating host cells, which comprise a large proportion of cells within the microenvironment of glioblastoma, to support their growth and maintain any immunosuppressive milieu.<sup>3</sup>

In addition to the soluble immunosuppressive molecule candidates mentioned above, immune-relevant molecules expressed at the cell surface of glioblastoma have attracted interest because there is major infiltration of glioblastomas by host cells, although the cells are mainly of macrophage-monocyte lineage. These surface molecules include the CD95 ligand, which may induce apoptosis in susceptible cells that express the receptor, CD95, previously referred to as Fas or APO-1. In the context of glioblastoma, cellular targets for CD95-mediated apoptosis are probably mainly T cells. Additional candidate cell surface molecules with immunosuppressive properties, but with uncertain significance in glioblastoma, include regeneration and tolerance factor (RTF), lectin-like transcript 1, and HLA-E and HLA-G.<sup>4</sup>

More recently, major emphasis has been placed on the aberrant expression of PD-L1 by glioblastoma cells. PD-L1 is the ligand for PD-1, a cell surface molecule expressed mainly on T cells now referred to as an immune checkpoint, together with CTLA-4. These two molecules, PD-1 and CTLA-4, mediate inactivation of T cells, and antibody-mediated therapeutic neutralization of these molecules with agents such as nivolumab or pembrolizumab that target PD-1, or ipilimumab that targets CTLA-4, can be considered the greatest innovation in medical oncology in the past decade.<sup>5</sup> They are currently being explored as novel agents across the full spectrum of human cancers and have already been integrated into the standard of care for malignant melanoma.

From The Johns Hopkins University, Baltimore, MD; University Hospital Zurich, Zurich, Switzerland; Institute for the Neurosciences at the Brigham and Women's/Faulkner Hospital, Center for Neuro-Oncology, Dana-Farber Cancer Institute, Boston, MA; The University of Chicago, Chicago, IL.

Disclosures of potential conflicts of interest provided by the authors are available with the online article at [asco.org/edbook](http://asco.org/edbook).

Corresponding author: Michael Lim, MD, Johns Hopkins Hospital, Neurosurgery-Phipps 123, 600 North Wolfe St., Baltimore, MD 21287; email: [mlim3@jhmi.edu](mailto:mlim3@jhmi.edu).

© 2016 by American Society of Clinical Oncology.

Furthermore, antibodies targeting the ligand PD-L1 rather than the receptor, such as atezolizumab, also are being explored in various tumor entities. The extent that glioblastomas express PD-L1 *in vivo* has remained controversial,<sup>6</sup> but PD-1 and CTLA-4 inhibition are remarkably active in mouse glioma models, both as single agents and in combination. Importantly, inhibition of immune checkpoints probably confers a nonspecific state of immune activation that will be of benefit to patients if their particular cancers carry a high mutational load, rendering them potentially recognizable as altered. Whether the observed benefit can be translated to glioblastoma remains to be clarified. Several clinical trial initiatives to explore immune checkpoint inhibitors in glioblastoma are now underway.

Because of disappointing results with traditional cancer therapy (radiotherapy, chemotherapy, and angiogenesis inhibition by promising agents, such as VEGF pathway inhibitors bevacizumab and cediranib) in this disease, various approaches to immunotherapy have gained a lot of interest. As of early 2016, several phase II and phase III clinical trials of immunotherapy are underway or have already been completed, including but not limited to (1) the rindopepimut vaccine that targets epidermal growth factor variant III, with a completed phase III trial in the newly diagnosed setting (ACT IV) and encouraging activity in phase II in the recurrent setting (Re-ACT); (2) the six-candidate peptide cocktail ICT-107, which is used to generate a vaccine by *ex vivo* stimulation of patient-derived dendritic cells, with a completed randomized phase II trial that indicated activity in patients who were HLA-A2 positive and a phase III program scheduled to start in early 2016; and (3) the DCVax phase III program that is near completion, with high logistical complexity—it uses autologous tumor to stimulate autologous dendritic cells.

These treatments likely are most effective with minimal residual tumor burden, which should be associated with decreased levels of soluble immunosuppressive factors and

decreased potential for immune cell re-education by bulky tumor. Accordingly, most recent immunotherapy trials have been and are now being conducted in highly selected patient populations in the newly diagnosed setting and among patients with little or no residual tumor after concomitant temozolomide chemoradiotherapy. Given these developments, it can be assumed that neurosurgery will be attributed a larger role not only in newly diagnosed patients but also in the setting of recurrent disease, if the first definitively positive immunotherapy data become available. Although removing the source of immunosuppression surgically seems to be the most straightforward approach to overcome immunosuppression, additional strategies include neutralizing TGF- $\beta$ , at least transiently, or employing immune adjuvants, such as granulocyte macrophage colony-stimulating factor or toll-like receptor 2 agonists. The most powerful combinatorial strategy for tumor-specific vaccines, however, is likely to be the checkpoint inhibitors, as outlined above. Such additional efforts at boosting immune responses may be essential for a benefit from upcoming strategies of immunotherapy in broader populations of patients, including patients with bulky disease, heavily pretreated patients, and older patients who are likely to exhibit impaired immune responsiveness.

## CHECKPOINT INHIBITORS

An important component of tumor-induced immunosuppression involves the costimulatory interaction. Normally, when a T cell encounters an antigen-presenting cell (APC) expressing the appropriate antigen, a second interaction with a checkpoint molecule is required to either activate or suppress the T cell (Fig. 2).<sup>7,8</sup> This second interaction plays an important role in modulating an immune response. Furthermore, there are multiple costimulatory molecules, which suggest that a hierarchy of activation status exists. In addition, this interaction is not unique to APCs and T cells; other immune cells, such as natural killer cells and regulatory T cells (Tregs), also have costimulatory molecules.<sup>8</sup> Hence, this suggests a hierarchy of immune cell activation as well as an ability to attenuate an immune response.

Checkpoint inhibitors are a class of antibodies that are designed to interrupt or activate these costimulatory molecules. Intense investigation is underway for the utilization of checkpoint inhibitors for the treatment of solid tumors. CTLA-4 was one of the first molecules to be studied. Leach et al<sup>9</sup> found that they could induce an antitumor immune response within a murine model for melanoma by using an anti-CTLA-4 antibody.<sup>9</sup> The second checkpoint inhibitor that has been intensely studied is anti-PD-1. Anti-PD-1 has been shown to induce an antitumor immune response in multiple solid tumors, including glioblastoma.<sup>10,11</sup>

## Proven Efficacy of Checkpoint Inhibitors

Several large clinical trials in humans verified the observed efficacy of checkpoint inhibitors in the preclinical models. In 2010, Hodi et al<sup>12</sup> ran a large phase III trial that demonstrated

### KEY POINTS

- Immunotherapy has proven efficacy for other solid tumors and is now a focus for glioblastoma.
- An important component of glioblastoma-mediated immunosuppression stems from soluble factors such as TGF- $\beta$ .
- Checkpoint inhibition probably confers a nonspecific state of immune activation that is a promising approach to glioblastoma, given the success of checkpoint inhibitors in melanoma, lung cancer, and renal cancers.
- Current strategies for biomarkers have focused on protein expression, immune cell characterization, and mutational burden.
- Tumor-selective viruses and viral-based vectors have shown increasingly promising results as easy-to-use immunostimulants.

improved survival among patients with melanoma who were treated with anti-CTLA-4. The investigators also found that a subset of patients were long-term survivors.<sup>12</sup> Topalian et al<sup>13</sup> then published their experience in treating multiple solid tumors with anti-PD-1, in which they found that anti-PD-1 improved survival for patients with melanoma, renal cell carcinoma, and non-small cell lung cancer. These and other important studies resulted in U.S. Food and Drug Administration (FDA) approval of checkpoint inhibitors. Checkpoint inhibitors first gained FDA approval for use in patients with melanoma in 2011, with the approval of anti-CTLA-4. The FDA approved anti-PD-1 in September 2014 for melanoma. Shortly after, anti-PD-1 was approved for lung cancer (both adenocarcinoma and squamous cell carcinoma) and renal cell cancer. Hence, the enthusiasm for the use of checkpoint inhibitors in glioblastoma is high.

### Checkpoint Inhibitors for Glioblastoma

There are promising preclinical data suggesting that checkpoint inhibitors may promote an antitumor immune response. Fecci et al<sup>14</sup> demonstrated improved survival mediated by a CD4<sup>+</sup> T-cell immune response in a murine glioma model treated with anti-CTLA-4. Zeng et al and others have shown that anti-PD-1 monotherapy improved survival in a murine model for glioblastoma. Interestingly, anti-PD-L1 alone did not result in much survival improvement. With anti-PD-1 use, the CD8<sup>+</sup> T cells appear to be responsible for the antitumor immune response.<sup>10,11</sup>

### CURRENT TRIALS FOR GLIOBLASTOMA

Because we observed improved survival with the use of checkpoint inhibitors in the previously mentioned solid tumors, these compounds are now being applied to glioblastoma. Several clinical trials are currently underway, with encouraging preclinical results.

#### NCT02017717

NCT02017717 is a large, randomized, phase III open-label trial sponsored by Bristol Myers Squibb for patients with a first-time recurrence of glioblastoma that used anti-PD-1 as the treatment backbone to assess its safety and, ultimately, its efficacy. The study began with a small safety run-in, during which patients were treated with anti-PD-1 alone or anti-PD-1 with anti-CTLA-4. Interim safety data that were presented at the 2015 American Society of Clinical Oncology Annual Meeting showed that the rates of severe adverse events were significantly higher among patients who received the combination of anti-PD-1 and anti-CTLA-4; 40% of patients had to discontinue therapy (10 patients per arm). Thus, a decision was made to expand the anti-PD-1 cohort and to use bevacizumab as the comparator arm. Although this was a study of safety, a partial response in one patient who was treated with anti-PD-1 alone, and a few cases of pseudoprogression, was observed. The trial has finished accrual.

#### NCT02337491

NCT02337491 is phase II trial based on the premise that anti-VEGF therapy could be synergistic with immunotherapy.<sup>15</sup> This trial is a single-institution study sponsored by Merck using Merck's anti-PD-1 drug. The study will measure progression-free survival at 6 months. The trial has two arms: anti-PD-1 alone and anti-PD-1 with bevacizumab.

#### NCT02336166

NCT02336166 is a phase II trial sponsored by the Ludwig Foundation to study the anti-PD-1 therapy developed by AstraZeneca. The trial has multiple objectives. The first cohort (cohort A) will study the efficacy of anti-PD-L1 for patients with newly diagnosed glioblastoma that has unmethylated O(6)-methylguanine DNA methyltransferase. The remarkable factor in this arm is that temozolomide (TMZ) will be withheld; patients will receive only anti-PD-L1 and radiation. The rationale behind this design is that radiation and anti-PD-L1 are synergistic, so systemic chemotherapy may be counterproductive. The second cohort (cohort B) will assess the efficacy of anti-PD-L1 alone for patients who have recurrent glioblastoma. The third cohort (cohort C) will administer anti-PD-L1 to patients who have recurrent glioblastoma that is progressing with bevacizumab treatment.

#### NCT02617589

NCT02617589 is a phase III trial sponsored by Bristol Myers Squibb to assess the efficacy of anti-PD-1 with radiation among patients who have newly diagnosed glioblastoma. The comparator arm will enroll patients for treatment with radiation and temozolomide (standard of care).

#### NCT02313272

NCT02313272 is a phase I trial to assess the safety of an anti-PD-1 drug developed by Merck with bevacizumab and hypofractionated stereotactic irradiation for patients who have recurrent high-grade gliomas.

#### NCT02530502

NCT02530502 is a phase I/II trial for patients with newly diagnosed glioblastoma. Phase I will assess the safety of combining an anti-PD-1 drug developed by Merck with radiation and temozolomide for patients with newly diagnosed glioblastoma. Phase II will compare the efficacy of adding anti-PD-1 to radiation and temozolomide for patients treated with radiation and temozolomide.

#### NCT02311582

NCT02311582 is a phase I, open-label, randomized safety study to assess the addition of MK-3475, an anti-PD-1 drug developed by Merck, for treatment among patients who are being treated with laser ablation for recurrent glioblastoma.

## BIOMARKERS

What we have learned from checkpoint inhibitor trials in other tumors is that the overall response rate is between 20% and 30%. Therefore, it is important to identify patients who would not respond and to minimize toxicities and treat them with other therapies. Current strategies for biomarkers have focused on protein expression, immune cell characterization, and mutational burden.

The melanoma and lung cancer trials have focused on the expression of PD-L1 on tumor cells as a biomarker to predict response. Investigators have found that tumors that express PD-L1 were more likely than PD-L1-negative tumors to respond (overall response rate) to anti-PD-1 therapy.<sup>16</sup> Interestingly, the expression of PD-L1 was not important in the trial of patients with melanoma who received the concurrent combination of anti-PD-1 and anti-CTLA-4. Some theorized that an adaptive immune response occurred with the combination therapy and that combination caused tumor cells to express PD-L1 as a defense mechanism.<sup>17</sup> In an interesting twist, when the therapies were sequenced—patients received anti-CTLA-4 first, followed by anti-PD-1—the overall response rate again correlated to the PD-L1 status of the tumor.<sup>18</sup>

Another approach has been to assess the activation status of various immune cells as a predictor of response. As an example, investigators assessed the activation status of CD8+ T cells by measuring eomesodermin for patients with melanoma after they received treatment with anti-CTLA-4 and found that the eomesodermin status predicted relapse-free survival. Other markers of interest are interferon gamma expression, Helios expression, and various other checkpoint molecules.<sup>19</sup>

Last, investigators have assessed mutational burden as a predictor of response. Chan et al<sup>20</sup> correlated mutational burden among patients with lung cancer to response rates among patients who received anti-PD-1 therapy. They found that patients with lung cancer who had a history of smoking had higher mutational burdens than patients with lung cancer who did not smoke and that the higher number of mutations correlated to improved survival.<sup>20</sup> Le et al<sup>21</sup> also studied patients with colon cancer and found that patients who had a defective DNA repair gene had a higher number of mutations in their tumor, and this again correlated to improved survival. Some theorize that the reason for the observed improved antitumor immune responses in patients with tumors that had more mutations was that the tumors expressed a higher number of target antigens for the immune system.<sup>21,22</sup>

## TOXICITY

Immune-related toxicities are an important issue for patients who receive immunotherapy. Most of the toxicities are related to autoimmune reactions. The most common toxicities include colitis, pneumonitis, hepatitis, pancreatitis, dermatitis, hypophysitis, and thyroiditis. If the toxicities are not recognized early, these reactions could become life

threatening. Treatments often require stopping the treatment, starting high-dose corticosteroids, and possibly administering infliximab.<sup>12,13,23</sup>

## IMAGING

Determining response to immunotherapy with imaging has become an area of intense interest. In the trial reported by Hodi et al,<sup>12</sup> a large number of patients with melanoma who received anti-CTLA-4 experienced pseudoprogression. Furthermore, the researchers found that pseudoprogression could take months to resolve.<sup>24,25</sup> As a result, many trials have built in lag times for imaging to allow patients to continue therapy and to avoid considering the treatment a failure. In glioblastoma, Okada et al<sup>26</sup> have developed an iRANO protocol specifically tailored for immunotherapy.

## COMBINATION THERAPY

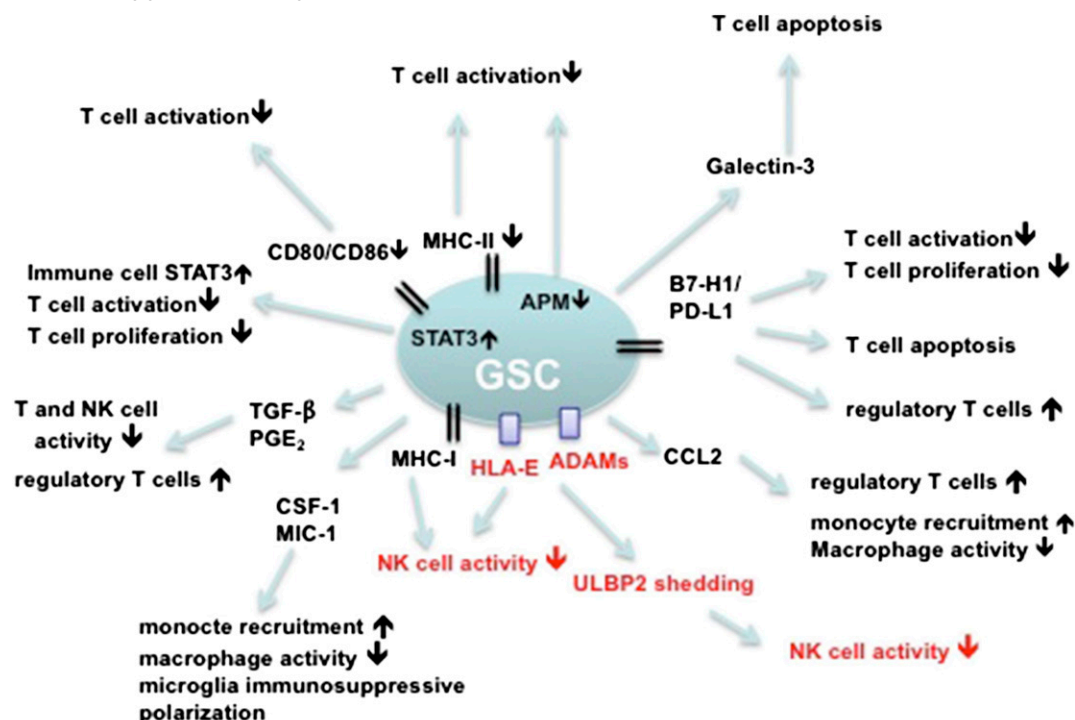
As previously mentioned, the response rates for patients who receive immunotherapy has ranged from 20% to 30%. Studies are investigating ways to combine checkpoint inhibitors with other modalities, such as radiation, bevacizumab, and devices. Zeng et al<sup>10</sup> demonstrated in a preclinical glioblastoma model that the combination of focused radiation with anti-PD-1 is synergistic. Clinical trials (e.g., NCT02313272) are looking at stereotactic radiation use in combination with checkpoint inhibitors. Bevacizumab also may work synergistically with immunotherapy.<sup>20</sup> Preclinical data suggest that this approach could be effective, and a phase II trial (NCT02337491) is looking at the combination of bevacizumab and anti-PD-1 for patients with glioblastoma. Finally, another interesting approach is the combination of laser ablation with anti-PD-1. A phase I trial (NCT02313272) is looking at the combination of anti-PD-1 with laser ablation.

In conclusion, checkpoint inhibitors have shown great promise in other solid tumors. There is much excitement about checkpoint inhibitors in the setting of glioblastoma. Several large trials are underway to assess the efficacy of checkpoint inhibitors in glioblastoma.

## VIRAL- AND GENE-MEDIATED IMMUNOTHERAPIES FOR GLIOBLASTOMA

We have discussed the various types of immunotherapy for glioblastoma. Now, we discuss one additional mode that involves the use of genetically engineered viruses to deliver cytotoxic/immunostimulatory genes into tumors.<sup>27</sup> Two main types of viruses are used in this technology: replication-defective vectors, in which viral genes have been removed so there is no expression of viral genes or generation of progeny viruses, but there is expression of an immunostimulatory and/or cytotoxic gene, and tumor replication-selective viruses (oncolytic viruses), in which a viral pathogen is engineered so that its pathogenicity is now targeted to tumor cells and not normal cells.<sup>28</sup> It is recognized now that the presence of viral genes and viral proteins in both of these technologies can elicit powerful anticancer immune responses, which are a major component of efficacy.<sup>29</sup>



**FIGURE 1. Immunosuppressive Properties of Glioma Stem Cells**

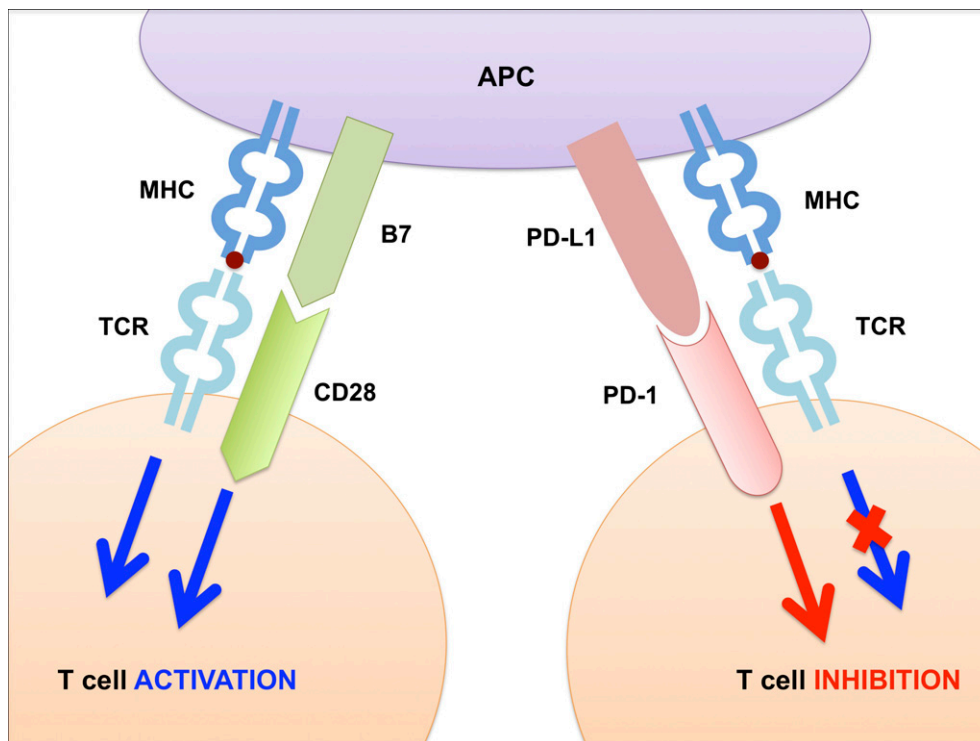
Abbreviation: TGF- $\beta$ , transforming growth factor-beta.  
Modified from Hatiboglu et al.<sup>42</sup>

Characteristics that differentiate this immunotherapy from others are the following: this immunotherapy involves a neurosurgical component of direct injection into glioblastoma, either by stereotaxy or free-hand injection during a craniotomy; is off-the-shelf, in that manipulation of cells from the patient before or after treatment is not involved; and does not require a priori knowledge of the identity of tumor-specific antigens that require targeting and, at least in theory, the cytotoxic action of the viral vector-delivered cytotoxic gene or of the replicating oncolytic viruses will expose the repertoire of all tumor-specific antigens to the immune system.

There are more than 2 decades of clinical trial experiences that used various types of replication-defective vectors to deliver various types of cytotoxic/immunostimulatory genes, but none have resulted in an FDA-approved clinical product, and two phase III clinical trials—a retroviral vector to deliver the cytotoxic/immunostimulatory herpes thymidine kinase (*TK*) gene for recurrent glioblastoma<sup>30</sup> and an adenoviral vector to deliver *TK* in newly diagnosed glioblastoma (ASPECT)—have failed.<sup>31</sup> However, we recently reported the results of a phase II trial in which aglatimagene besadenovec (AdV-tk), a non-replicating adenovirus expressing the herpesvirus *TK* gene, was free-hand injected in a resected, newly diagnosed, malignant glioma cavity.<sup>32,33</sup> The patient then received the oral antiherpetic prodrug (valacyclovir) while undergoing standard-of-care radiochemotherapy (gene-mediated cytotoxic immunotherapy [GMCI]). There are multiple modes of cytotoxic and immunostimulatory anticancer action (Fig. 3):

1. The delivered *TK* gene product phosphorylates the administered valacyclovir drug, which becomes incorporated at sites where DNA is becoming repaired or where DNA is replicating. This leads to termination of DNA repair and/or replication, leading to immunogenic cell death.
2. Standard of care also leads to cytotoxicity, and radiation-induced DNA damage leads to additional unsuccessful and cytotoxic attempts at DNA repair using the phosphorylated valacyclovir pools.
3. The delivered *TK* antigen and viral vector proteins act as superantigens, providing an immunostimulatory stimulus in the glioblastoma microenvironment.
4. The cytotoxic death of glioblastoma cells is also immunogenic, releasing and exposing multiple glioma antigens to the immune system.

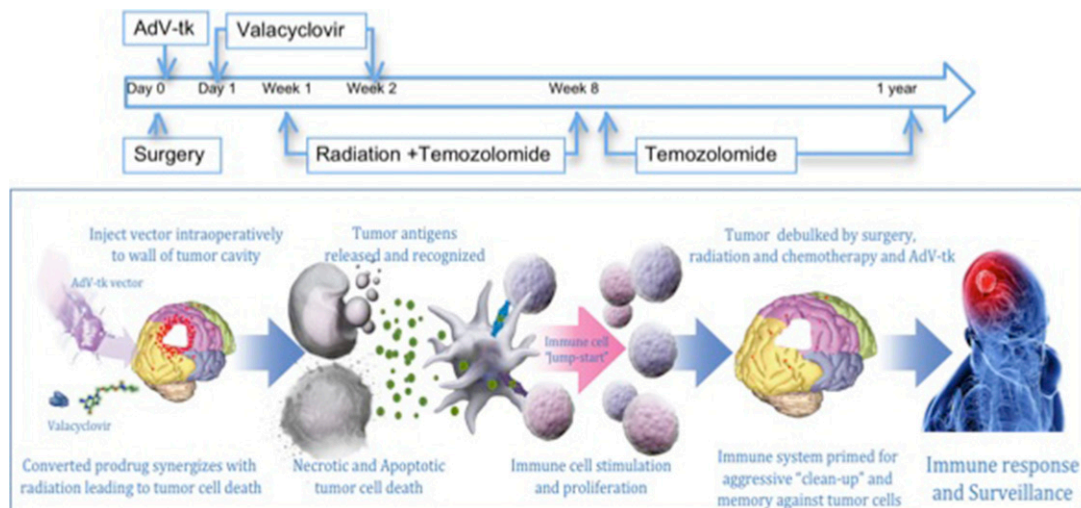
Our published mature phase II data appear to show encouraging, albeit not definite, results in terms of possible efficacy on the basis of the extent of residual tumor burden: the median overall survival (OS) durations for patients who underwent gross total resection were 25.0 and 16.9 months (a difference of 8.1 months) for GMCI/standard of care and standard of care, respectively (hazard ratio 0.59; 95% CI, 0.35–0.998;  $p = .0492$ ); for patients who underwent subtotal resections, the difference was only 1 month (13.5 vs. 12.5 months for GMCI/standard of care vs. standard of care;  $p = .4584$ ).<sup>33</sup> To further improve this therapy, we have returned to the laboratory. The current theory is that to be effective, an anticancer immune response by cytotoxic

**FIGURE 2. Examples of Activating (CD28) and Inhibiting (PD-1) Immune Checkpoints**

T cells requires removal of immune checkpoint signaling mediated by PD-1/PD-L1,<sup>34-36</sup> the CTLA-4/B7 family,<sup>37,38</sup> and other molecules. We thus hypothesize that a combination of GMCI and checkpoint inhibitors may lead to more effective immunotherapy.<sup>8</sup> In fact, we are finding that the application of GMCI in mouse gliomas does lead to increase signaling of immune checkpoint networks and that inhibition of these

networks does lead to even more encouraging antiglioma effects of GMCI.

For the second type of viral-mediated therapy, multiple types of oncolytic viruses have been tested in clinical trials of glioblastoma, all in the recurrent setting. No trial for glioblastoma has progressed to phase III, but there are two oncolytic viruses (an oncolytic adenovirus from DNatrix

**FIGURE 3. Schematics of the Published Clinical Trials and Different Modes of Anticancer Action of Gene-Mediated Cytotoxic Immunotherapy**

(Top panel) Schematic of the published clinical trials of gene-mediated cytotoxic immunotherapy (GMCI). On the day of surgery, the adenoviral vector that delivers TK (AdV-tk) is injected in the resected newly diagnosed glioblastoma tumor cavity. The oral agent (valacyclovir) is administered to the patient on days 1 to 14. Standard-of-care radiation and temozolomide are also administered as per the Stupp regimen. (Lower panel) Schematic of the different modes of anticancer action of GMCI.

and an oncolytic retrovirus from Tocagen) that have completed phase II evaluation and planning for advanced phase III trials. Commercial interest in this area of oncolytic virus-based immunotherapy has been resurrected recently by the FDA approval of a herpes simplex virus (HSV) oncolytic virus for melanoma.<sup>29,39</sup> Our group has been involved in the preclinical and clinical development of an oncolytic virus, based on HSV1, that has been engineered to selectively replicate and destroy gliomas on the basis of tumor deregulation of the p16 tumor suppressor pathway and expression of the glioma stem-cell marker, nestin.<sup>40,41</sup> This oncolytic virus (rQNestin34.5v.2) shows potency in animal models of gliomas compared with older, clinical trial–tested versions of herpes oncolytic viruses. Extensive preclinical data

have been obtained to justify filing of an investigational new drug application with the FDA for a planned first-in-human clinical trial among patients with recurrent glioblastoma.

In summary, the use of tumor-selective viruses and viral-based vectors is increasingly delivering promising results as an easy to use immunostimulant approach, but only the successful completion of phase III trials for several of these products will show if the treatments have achieved their promise as anticancer agents or if additional laboratory development is required.

## ACKNOWLEDGMENT

We thank Eileen Kim for her help with Fig. 2.

## References

- Roszman T, Elliott L, Brooks W. Modulation of T-cell function by gliomas. *Immunol Today*. 1991;12:370-374.
- Frei K, Gramatzki D, Tritschler I, et al. Transforming growth factor- $\beta$  pathway activity in glioblastoma. *Oncotarget*. 2015;6:5963-5977.
- Nduom EK, Weller M, Heimberger AB. Immunosuppressive mechanisms in glioblastoma. *Neuro Oncol*. 2015;17:vii9-vii14.
- Weiss T, Weller M, Roth P. Immunotherapy for glioblastoma: concepts and challenges. *Curr Opin Neurol*. 2015;28:639-646.
- Preusser M, Lim M, Hafler DA, et al. Prospects of immune checkpoint modulators in the treatment of glioblastoma. *Nat Rev Neurol*. 2015;11:504-514.
- Preusser M, Berghoff AS, Wick W, et al. Clinical neuropathology mini-review 6-2015: PD-L1—emerging biomarker in glioblastoma? *Clin Neuropathol*. 2015;34:313-321.
- Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell*. 2015;161:205-214.
- Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252-264.
- Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 1996;271:1734-1736.
- Zeng J, See AP, Phallen J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. *Int J Radiat Oncol Biol Phys*. 2013;86:343-349.
- Reardon DA, Freeman G, Wu C, et al. Immunotherapy advances for glioblastoma. *Neuro Oncol*. 2014;16:1441-1458.
- Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363:711-723.
- Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366:2443-2454.
- Fecci PE, Ochiai H, Mitchell DA, et al. Systemic CTLA-4 blockade ameliorates glioma-induced changes to the CD4<sup>+</sup> T cell compartment without affecting regulatory T-cell function. *Clin Cancer Res*. 2007;13:2158-2167.
- Hodi FS, Lawrence D, Lezcano C, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. *Cancer Immunol Res*. 2014;2:632-642.
- Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res*. 2014;20:5064-5074.
- Grosso J, Horak CE, Inzunza D, et al. Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients (pts) with advanced solid tumors treated with nivolumab (anti-PD-1;BMS-936558;ONO-4538). *J Clin Oncol*. 2013;31(suppl; abstr 3016).
- Callahan MK, Postow MA, Wolchok JD. CTLA-4 and PD-1 pathway blockade: combinations in the clinic. *Front Oncol*. 2014;4:385.
- Wang W, Yu D, Sarnaik AA, et al. Biomarkers on melanoma patient T cells associated with ipilimumab treatment. *J Transl Med*. 2012;10:146.
- Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology: mutational landscape determines sensitivity to PD-1 blockade in non-small-cell lung cancer. *Science*. 2015;348:124-128.
- Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509-2520.
- Diaz LA Jr, Le DT. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;373:1979.
- Weber JS, Kähler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. *J Clin Oncol*. 2012;30:2691-2697.
- Hodi FS, Ribas A, Daud A, et al. Patterns of response in patients with advanced melanoma treated with pembrolizumab (MK-3475) and evaluation of immune-related response criteria (irRC). *J Immunother Cancer*. 2014;2:P103.
- Topalian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol*. 2014;32:1020-1030.
- Okada H, Weller M, Huang R, et al. Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. *Lancet Oncol*. 2015;16:e534-e542.
- Kaufmann JK, Chiocca EA. Glioma virus therapies between bench and bedside. *Neuro Oncol*. 2014;16:334-351.
- Chiocca EA, Rabkin SD. Oncolytic viruses and their application to cancer immunotherapy. *Cancer Immunol Res*. 2014;2:295-300.
- Lawler SE, Chiocca EA. Oncolytic virus-mediated immunotherapy: a combinatorial approach for cancer treatment. *J Clin Oncol*. 2015;33:2812-2814.
- Rainov NG. A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum Gene Ther*. 2000;11:2389-2401.



31. Westphal M, Ylä-Herttuala S, Martin J, et al; ASPECT Study Group. Adenovirus-mediated gene therapy with sitimagene ceradenovec followed by intravenous ganciclovir for patients with operable high-grade glioma (ASPECT): a randomised, open-label, phase 3 trial. *Lancet Oncol*. 2013;14:823-833.
32. Chiocca EA, Aguilar LK, Bell SD, et al. Phase IB study of gene-mediated cytotoxic immunotherapy adjuvant to up-front surgery and intensive timing radiation for malignant glioma. *J Clin Oncol*. 2011;29:3611-3619.
33. Wheeler LA, Manzanera AG, Bell SD, et al. Phase 2 multicenter study of gene-mediated cytotoxic immunotherapy as adjuvant to surgical resection for newly diagnosed malignant glioma. *Neuro Oncol*. Epub 2016 Feb 2.
34. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 2006;439:682-687.
35. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000;192:1027-1034.
36. Mahoney KM, Rennert PD, Freeman GJ. Combination cancer immunotherapy and new immunomodulatory targets. *Nat Rev Drug Discov*. 2015;14:561-584.
37. Lane P. Regulation of T and B cell responses by modulating interactions between CD28/CTLA4 and their ligands, CD80 and CD86. *Ann N Y Acad Sci*. 1997;815:392-400.
38. Korman A, Yellin M, Keler T. Tumor immunotherapy: preclinical and clinical activity of anti-CTLA4 antibodies. *Curr Opin Investig Drugs*. 2005;6:582-591.
39. Andtbacka RH, Kaufman HL, Collichio F, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J Clin Oncol*. 2015;33:2780-2788.
40. Aghi M, Visted T, Depinho RA, et al. Oncolytic herpes virus with defective ICP6 specifically replicates in quiescent cells with homozygous genetic mutations in p16. *Oncogene*. 2008;27:4249-4254.
41. Kambara H, Okano H, Chiocca EA, et al. An oncolytic HSV-1 mutant expressing ICP34.5 under control of a nestin promoter increases survival of animals even when symptomatic from a brain tumor. *Cancer Res*. 2005;65:2832-2839.
42. Hatiboglu MA, Wei J, Wu AS, et al. Immune therapeutic targeting of glioma cancer stem cells. *Target Oncol*. 2010;5:217-227.